LIQUID-DIPPED SCANNING PROBE MICROSCOPE DEVICE

Patent Number: JP9119939

Publication date: 1997-05-06

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Requested Patent: JP9119939

Application Number: JP19950277924 19951025

Priority Number(s):

IPC Classification: G01N37/00; G01B21/30; H01J37/28

EC Classification:

Equivalents:

Abstract

PROBLEM TO BE SOLVED: To provide a liquid dipped scanning type probe microscope with less adhesion of air bubble to the surface of a member which is dipped in a solution.
SOLUTION: A probe 20, a cantilever 22, the entire portion of a window member 30, and one portion of a cantilever retention member 24 are sunk in a solution 16. After that, an interface activator is supplied onto the liquid surface of the solution 16 by a micro dispenser 32. Then, a head 36 is raised to such a height that the cantilever 22 and the probe 20 do not touch the activator on the liquid surface, thus adhering the interface activator to the cantilever retention member 24 and the window member 30. After that, the cantilever holding member 24 is sunk in the solution again. At this time, since the interface activator adhered to the cantilever holding member 24 and the window member 30 properly adapts itself to the interface activator on the liquid surface, no air bubble is generated on the surface. Finally, the interface activator remaining on the liquid surface is sucked by a collecting means 34.

(19)日本国特許庁（ＪＰ）　(12) **公開特許公報**（Ａ）　(11)特許出願公開番号

特開平9－119939

(43)公開日　平成９年(1997)５月６日

(51)Int.Cl.[6]	識別記号	庁内整理番号	ＦＩ		技術表示箇所
Ｇ０１Ｎ　37/00			Ｇ０１Ｎ　37/00	Ｆ	
Ｇ０１Ｂ　21/30			Ｇ０１Ｂ　21/30	Ｚ	
Ｈ０１Ｊ　37/28			Ｈ０１Ｊ　37/28	Ｚ	

審査請求　未請求　請求項の数２　ＯＬ　（全７頁）

(21)出願番号　特願平7－277924

(22)出願日　平成７年(1995)10月25日

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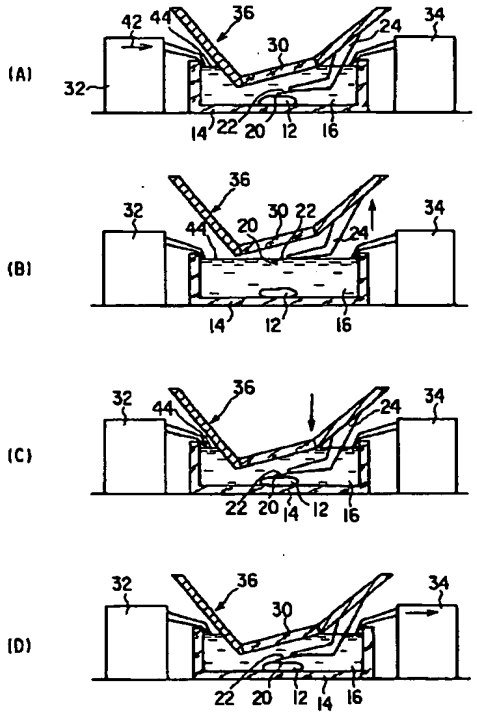
(54)【発明の名称】　液浸走査型プローブ顕微鏡装置

(57)【要約】

【課題】溶液中に浸る部材の表面に対する気泡の付着が少ない液浸走査型プローブ顕微鏡を提供する。

【解決手段】探針２０とカンチレバー２２と窓部材３０の全体とカンチレバー保持部材２４の一部を溶液１６に沈める。その後、マイクロディスペンサー３２により溶液１６の液面上に界面活性剤を供給する（Ａ）。次に、カンチレバー２２と探針２０が液面上の界面活性剤に触れない高さまでヘッド３６を上昇させる（Ｂ）。これによりカンチレバー保持部材２４と窓部材３０には界面活性剤が付着する。続いて、カンチレバー保持部材２４を再び溶液中に沈める（Ｃ）。このとき、カンチレバー保持部材２４と窓部材３０に付着した界面活性剤は液面上の界面活性剤と良くなじむため、これらの表面に気泡は生じない。最後に、回収手段３４を用いて液面上に残る界面活性剤を吸引する（Ｄ）。



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【特許請求の範囲】

【請求項１】容器に入れた溶液中に配置された試料を探針を用いて観察する液浸走査型プローブ顕微鏡において、

親水化処理された、先端に探針を備えたカンチレバーと、

カンチレバーを保持するカンチレバー保持部材と、

カンチレバーおよびカンチレバー保持部材を溶液中に浸入待避させる手段と、

溶液の液面上に界面活性剤を供給する手段とを備えており、

試料の観察に先立ち、

カンチレバーの全体とカンチレバー保持部材を溶液中に沈め、

溶液の液面上に界面活性剤を供給して液面上に界面活性剤の薄膜を形成し、

カンチレバーが界面活性剤に触れない範囲でカンチレバー保持部材をなるべく引き上げ、

再びカンチレバー保持部材を下げることを特徴とする液浸走査型プローブ顕微鏡。

【請求項２】請求項１において、

カンチレバーの変位を光学的に検出する変位検出系と、

変位検出系を溶液から保護するために変位検出系を収容する筐体とを備え、

筐体は、変位検出系とカンチレバーとの間での光の往来を許す光学的に透明な窓部材を有し、窓部材は液面に対して斜めに配置されており、

試料の観察に先立ち、

窓部材を溶液中に沈め、

溶液の液面上に界面活性剤を供給して液面上に界面活性剤の薄膜を形成し、

窓部材を引き上げ、

再び窓部材を溶液中に沈めることを特徴とする液浸走査型プローブ顕微鏡。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は走査型プローブ顕微鏡装置に関する。

【０００２】

【従来の技術】走査型プローブ顕微鏡は、試料の走査中に表面の形状に沿って追従する探針の挙動を検出することによって、試料の微細な形状を観察する装置であり、探針を追従させる手段の種別によって、走査型トンネル顕微鏡（ＳＴＭ）、原子間力顕微鏡（ＡＦＭ）、磁気力顕微鏡（ＭＦＭ）などが知られている。

【０００３】例えば、原子間力顕微鏡（ＡＦＭ）では、試料表面を走査する探針は柔軟なカンチレバーに支持され、カンチレバーまたは試料は圧電体等の微動素子を有する微動変位調整装置に支持され、探針と試料の間隔が調整可能となっている。探針を試料表面に近づけると、

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探針先端の原子と試料表面の原子の間に、ファンデルワールス相互作用による引力が働き、さらに原子の相互距離程度にまで近づけると、今度はパウリの排他律による斥力が働く。これらの引力と斥力は総称して原子間力と呼ばれる。原子間力は極めて微弱だが、探針を支持しているカンチレバーは、非常に柔軟であるため、探針先端の原子が原子間力を受けると、その大きさに応じて変位する。微動変位調整装置は、カンチレバーの変位量を一定に保つように、探針と試料の間隔をフィードバック制御する。このフィードバック制御時に圧電体等の微動素子に印加する電圧は、探針が走査している試料表面の形状に対応しているので、この印加電圧情報より試料表面の高さ情報が得られ、これを試料表面上の位置情報と併せて処理することで試料表面の凹凸像が得られる。

【０００４】この走査型プローブ顕微鏡装置を用いて水溶液中に保存した細胞等の生体試料を観察できれば、電子顕微鏡のような試料の固定や真空下での観察とは異なり、生きたままの状態で、試料の形状や、探針に加わる力の解析による表面の物性の計測などを数ｎｍの分解能で行なうことが可能となるため、溶液中で使用可能な走査型プローブ顕微鏡装置の開発が色々と試みられている。

【０００５】その代表的な例として米国特許４９３５６３４号に開示されている走査型プローブ顕微鏡を図５と図６に示す。図５は走査型プローブ顕微鏡の全体の構成を示す断面図であり、図６（Ａ）は図５の探針保持部を下から見た図、図６（Ｂ）は探針保持部の断面図である。

【０００６】図５に示すように、カンチレバー１０５を支持する探針保持部材１０１と、圧電体１０２上に設けられた試料台１０３は、両者の間に配置されたリング状の部材１０４に接触しており、これらの部材に囲まれた空間が水溶液で満たされる。図６に示すように、リング状の部材１０４の内側に位置する探針保持部材１０１の天井部分には、溶液を送り込むための供給管１１０と排出するための排出管１１１が設けられている。これにより、細胞等の生体試料を浸漬し、生きたまま保存するための酸素の供給や溶液の温度調整を可能にしている。図５に戻り、試料台１０３上に置かれた試料は圧電体１０２によって移動され、これによりカンチレバー１０５の先端に設けられた探針が走査される。走査の間、探針の変位は、カンチレバー１０５の上面に光を照射するレーザー光源１０６と、その反射光を受ける受光素子１０７とで構成される変位検出系により検出される。

【０００７】

【発明が解決しようとする課題】液浸用走査型プローブ顕微鏡装置では、測定の際には、上の例の様に所定の空間に溶液を注入したり、あるいは、探針を備えるカンチレバーを試料容器に溜めた溶液の中に沈めたりする。このとき、カンチレバーやカンチレバー保持部材の表面に

気泡が付着することがある。いったん発生してしまった気泡は取り除くことが難しい。このような気泡は、走査の障害となり、また同時に使用される顕微鏡観察の障害となり好ましくない。

【０００８】探針とカンチレバーに関しては、親水化処理を施すことにより、気泡の発生を防止することができる。親水化処理は、例えば、窒化シリコン製のカンチレバーに対して、酸化プラズマ処理を施したり、紫外線を照射したりすることにより行なえる。しかし、カンチレバー保持部材は一般に金属で作られるため、親水化処理を施すことができない。

【０００９】水溶液中の部材の表面への気泡の付着を防ぐ方法として、水溶液中に界面活性剤を混入することが一般に知られているが、観察対象である標本が生体試料である場合には、水溶液中への界面活性剤の混入は、試料の保存の都合上好ましくない。

【００１０】また、液浸用走査型プローブ顕微鏡装置には光てこ方式の変位検出系を用いたものがあり、この場合、防水の目的で、光学的に透明な防水用窓部材がカンチレバーと変位検出系との間に配置されるのが一般的である。この防水用窓部材に対しても、溶液を注入する際や探針を沈める際に、気泡が付着することがあり、この気泡もやはり取り除くことが難しい。防水用窓に付着した気泡は、光てこ方式の変位検出系におけるカンチレバーに向かうレーザー光やカンチレバーからの反射光を散乱させたりする。これは、変位検出精度の低下の原因となり好ましくない。また、同時に使用される光学顕微鏡による観察にとっても障害となり好ましくない。本発明の目的は、溶液中に浸る部材の表面に対する気泡の付着が少ない液浸走査型プローブ顕微鏡を提供することである。

【００１１】

【課題を解決するための手段】本発明は、容器に入れた溶液中に配置された試料を探針を用いて観察する液浸走査型プローブ顕微鏡において、親水化処理された、先端に探針を備えたカンチレバーと、カンチレバーを保持するカンチレバー保持部材と、カンチレバーおよびカンチレバー保持部材を溶液中に浸入待避させる手段と、溶液の液面上に界面活性剤を供給する手段とを備えており、試料の観察に先立ち、カンチレバーの全体とカンチレバー保持部材を溶液中に沈め、溶液の液面上に界面活性剤を供給して液面上に界面活性剤の薄膜を形成し、カンチレバーが界面活性剤に触れない範囲でカンチレバー保持部材をなるべく引き上げ、再びカンチレバー保持部材を下げることを特徴とする。

【００１２】さらに、上記の構成において、カンチレバーの変位を光学的に検出する変位検出系と、変位検出系を溶液から保護するために変位検出系を収容する筐体とを備え、筐体は、変位検出系とカンチレバーとの間での光の往来を許す光学的に透明な窓部材を有し、窓部材は

液面に対して斜めに配置されており、試料の観察に先立ち、窓部材を溶液中に沈め、溶液の液面上に界面活性剤を供給して液面上に界面活性剤の薄膜を形成し、窓部材を引き上げ、再び窓部材を溶液中に沈めることを特徴とする。

【００１３】

【発明の実施の形態】以下、図面を参照しながら本発明の実施の形態について説明する。

＜第一の実施の形態＞まず、第一の実施の形態について図１～図３を用いて説明する。

【００１４】図１（Ａ）に示すように、試料１２は容器１４の底部に置かれ、容器１４には試料１２を浸漬するための溶液１６が満たされる。容器１４は機械式のＸＹステージ１８の上に置かれ、探針２０に対する試料１２の位置決めを行なうことができる。ＸＹステージ１８の上には、容器１４の両側に、溶液１６の液面に界面活性剤を供給する手段であるマイクロディスペンサー３２と、液面に供給された界面活性剤を回収する回収手段３４とが設けられている。

【００１５】図１（Ｂ）に示すように、探針２０はカンチレバー２２の先端に設けられており、カンチレバー２２はカンチレバー保持部材２４に取り付けられ支持される。探針２０とカンチレバー２２は、材質に窒化シリコンを使用し、１０秒間程度の酸化プラズマ処理を施すことによって、接触角１０度以下の親水性が達成されている。窒化シリコンの表面の親水処理は紫外線照射によっても得られる。カンチレバー保持部材２４は、その断面形状を図１（Ｃ）に示すように、上面２４ａと下面２４ｂはＶ字形状となっている。カンチレバー保持部材２４は、図１（Ａ）に示すように、ヘッド３６に着脱可能に取り付けられていて、カンチレバー２２を交換する際の作業性向上が図られている。また、カンチレバー保持部材２４の先端部のカンチレバー２２の近傍に圧電素子を利用した励磁手段を取り付けて、カンチレバー２２を一定の振幅で振動させながら試料に外力を加えることのなく測定する、いわゆるＡＣモードのＡＦＭ測定を行なってもよい。

【００１６】ヘッド３６は、カンチレバー２２の上方に位置する部分に光学的に透明な窓部材３０が斜めに取り付けられており、全体は密閉されていて溶液が浸入しないようになっている。ヘッド３６の内部には、カンチレバー２２の上面にレーザービームを照射するレーザー光源２６と、カンチレバー２２の上面からの反射光を受ける受光素子２８が収容されている。受光素子２８は入射光量に応じた出力を示す複数の受光領域を有しており、カンチレバー２２の変位は受光素子２８の受光領域上に形成される反射光のスポットの位置から求められる。

【００１７】ヘッド３６は円筒型圧電体３８によって支持されており、円筒型圧電体３８は昇降手段４０に取り付けられている。ヘッド３６は円筒型圧電体３８によっ

てＸＹ走査およびＺ位置制御のための水平方向および垂直方向の移動が可能となっている。また、ヘッド３６は昇降手段４０により上下に粗動可能となっており、例えば、容器１４を交換する際には容器１４を出し入れするための空間を確保するために上方に待避される。

【００１８】以下、図２と図３を用いて、カンチレバー２２およびカンチレバー保持部材２４を溶液１６に沈める手順について説明する。まず、昇降手段４０を用いてヘッド３６を下降させて、図２（Ａ）に示すように、探針２０とカンチレバー２２と窓部材３０の全体とカンチレバー保持部材２４の一部を溶液１６に沈める。探針２０とカンチレバー２２は親水化処理されているので、これらに気泡が付着することはない。その後、マイクロディスペンサー３２を用いて所定量の界面活性剤を溶液１６の液面上に供給し、液面に界面活性剤４２の単分子膜４４を形成する。供給する界面活性剤４２の量は、液面に単分子膜を形成するのに必要な量であり、容器１４の開口部の面積により決まる。このとき、液面の界面活性剤の各分子４６は、図３（Ａ）に示すように、溶液１６側に親水基４６ａを、大気側に疎水基４６ｂを向けて液面に並ぶ。使用する界面活性剤としては例えばステアリン酸バリウムがあげられる。

【００１９】次に、昇降手段４０を用いて、図２（Ｂ）に示すように、カンチレバー２２と探針２０が界面活性剤の単分子膜４４に触れない高さまでヘッド３６を上昇させる。このとき、窓部材３０の全体とカンチレバー保持部材２４の殆どの部分が大気中に引き上げられる。カンチレバー保持部材２４が溶液１６から引き上げられる際、液面上の界面活性剤の分子４６は、図３（Ｂ）に示すように、カンチレバー保持部材２４の表面に親水基４６ａを向けて付着する。図にはカンチレバー保持部材２４のみが示されているが同様に窓部材３０の表面にも親水基４６ａを向けて付着する。この結果、カンチレバー保持部材２４と窓部材３０の表面には、疎水基４６ｂが大気中に向いた界面活性剤の被膜４８が形成される。カンチレバー保持部材２４の上面と下面は図１（Ｃ）に示したようにＶ字形状となっているので、上面と下面にも界面活性剤の被膜４８が良好に形成される。このとき、探針２０とカンチレバー２２は親水化処理が施されているので、これらの表面には界面活性剤の被膜が形成されない様に注意することが肝要である。

【００２０】なお、図２に示されるようにカンチレバー保持部材２４は、カンチレバー２２の先端部から基端部方向に徐々に溶液１６に浸入するように溶液１６の液面に対して傾斜して構成することが好ましい。このように構成することにより、カンチレバー２２を界面活性剤に触れさせずにカンチレバー保持部材２４を有効に親水化処理することができる。

【００２１】続いて、昇降手段４０を用いてヘッド３６を下降させて、図２（Ｃ）に示すように、探針２０を試料１２の表面近くまで近づける。このとき、カンチレバー保持部材２４と窓部材３０の表面には外側に疎水基４６ｂが向いた界面活性剤の被膜が形成されており、液面には大気側に疎水基４６ｂを向けた界面活性剤の単分子膜があるので、カンチレバー保持部材２４と窓部材３０が再び溶液中に沈む際に、図３（Ｃ）に示すように、液面上の界面活性剤はカンチレバー保持部材２４と窓部材３０の表面の界面活性剤の被膜に良好に付着し、外側に親水基４６ａが向いた二層目の界面活性剤の被膜が形成される。この結果、カンチレバー保持部材２４と窓部材３０は表面に気泡が付着することなく溶液中に沈められる。

【００２２】最後に、図２（Ｄ）に示すように、回収手段３４を用いて、液面に接したノズルから液面上に残る界面活性剤を吸引する。この結果、図３（Ｄ）に示すように、界面活性剤は、カンチレバー保持部材２４と窓部材３０および溶液中に浸っている部材の周辺を除いて回収される。これで、ＡＦＭ測定の準備が完了する。

【００２３】＜第二の実施の形態＞次に、第二の実施の形態について図４を用いて説明する。ＸＹステージ１８の上には、二個の圧電体５２によってＸＹ方向に移動される微動ステージ５４が設けられており、試料１２と溶液１６を入れた容器１４は微動ステージ５４の上に配置される。

【００２４】走査の際、試料１２は微動ステージ５４によってＸＹ方向に移動される。従って、円筒型圧電体３８は、ヘッド３６をＺ方向に移動させるための伸縮動作を行なうだけで、これをＸＹ方向に移動させるための湾曲動作は行なわない。

【００２５】他の構成および作用は前述の第一の実施の形態と同じである。この実施の形態では、円筒型圧電体３８はＸＹ方向への移動のための湾曲動作が不要なので、その外径を大きくすることが許される。円筒型圧電体の外径を大きくすることにより、光学顕微鏡用の観察に有利な開口数の大きな大型のコンデンサレンズ等を内側に配置することが可能である。

【００２６】本発明は、上述の実施の形態に何等限定されるものではない。発明の要旨を逸脱しない範囲で行なわれる実施は、すべて本発明に含まれる。本明細書には以下の各項に記した発明が含まれている。

【００２７】１．　例えば生理食塩水等の溶液が満たされたシャーレ等の容器内に固定された試料表面を、溶液中に挿入した探針保持部材に締結された探針によって走査することにより試料の形状等の情報を得る液浸走査型プローブ顕微鏡装置において、水に不溶性の界面活性効果を有する物質（界面活性剤）の単分子膜を、容器に満たした溶液の液面上に形成し、探針を溶液中に導入する際にカンチレバー保持部材の表面に界面活性剤自身の吸着性を利用して被膜を形成することを特徴とする液浸走査型プローブ顕微鏡装置。

【００２８】［効果］　溶液中に導入した探針保持部材の表面に界面活性剤の分子が、親水基を外側に向けて被膜を形成することにより、液中に導入される探針保持部材の表面の気泡の発生を低減できるため、安定した観察操作が可能な走査型プローブ顕微鏡装置が提供される。

【００２９】２．　第１項において、カンチレバー保持部材の表面に界面活性剤の被膜を形成させる過程で、一度大気中から溶液中に導入した探針保持部材のみ液面より引き上げ、かかる後、再度、溶液中に導入することによって、カンチレバー保持部材表面に親水性の被膜を形成する動作プロセスを有することを特徴とする液浸走査型プローブ顕微鏡装置。

【００３０】［効果］　探針先端には界面活性剤分子が付着せず、探針先端への異物の付着を原因とする走査画像の画質の劣化が防止される。

３．　第１項において、カンチレバー保持部材の断面形状の上面と下面がＶ字形状を有することを特徴とする液浸走査型プローブ顕微鏡装置。

【００３１】［効果］　カンチレバー保持部材の上面と下面の断面形状がＶ字型であるため、溶液の液面上に形成された界面活性剤の薄膜面を通過する際の保持部材表面への界面活性剤の被膜形成が容易となり、界面活性剤による気泡発生の低減効果の優れた走査型プローブ顕微鏡装置が得られる。

【００３２】４．　第１項ないし第３項において、界面活性剤を溶液の液面上に供給する手段を有することを特徴とする液浸走査型プローブ顕微鏡装置。

［効果］　界面活性剤の供給手段を備えているので、ディスペンサー等の供給手段を別途に準備する必要がなく、溶液の液面上に界面活性剤の薄膜を観察操作の都度容易に形成することができる、操作性に優れた走査型プローブ顕微鏡装置が得られる。

【００３３】５．　第１項ないし第４項において、溶液の液面上の界面活性剤を回収する手段を有することを特徴とする液浸走査型プローブ顕微鏡装置。

［効果］　界面活性剤の回収手段を備えているので、溶液の液面上に形成された界面活性剤の薄膜を回収することができ、観察中の生体試料の保存のために溶液を還流させる場合には、液面上の界面活性剤が試料に達する可能性が低減される。また、界面活性剤にステアリン酸バリウム等の水に不溶な物質を使用して溶液中への混入を防止しているため、試料保護性に優れた走査型プローブ顕微鏡装置が得られる。

【００３４】６．　水に不溶性の界面活性効果を有する物質（界面活性剤）の単分子膜を、容器に満たした溶液の液面上に形成し、探針を溶液中に導入する際に、カンチレバーの変位を検出するためにカンチレバー上方に配される例えば光てこ方式等の変位検出手段の保護の目的でカンチレバーと変位検出手段の間に配される防水用窓部材の表面に界面活性剤自身の吸着性を利用して被膜を

形成することを特徴とする液浸走査型プローブ顕微鏡装置。

【００３５】［効果］　溶液中に導入した窓部材の表面に界面活性剤の分子が、親水基を外側に向けて被膜を形成することにより、液中に導入される窓部材の表面の気泡の発生を低減できるため、正確な変位検出が可能な走査型プローブ顕微鏡装置が提供される。

【００３６】７．　第６項において、防水用窓部材の表面に界面活性剤の被膜を形成させる過程で、一度大気中から溶液中に導入した探針保持部材のみ液面より引き上げ、かかる後、再度、溶液中に導入することによって、防水用窓部材の表面に親水性の被膜を形成する動作プロセスを有することを特徴とする液浸走査型プローブ顕微鏡装置。

【００３７】［効果］　窓部材に付着した界面活性剤と液面上の界面活性剤は良くなじむので、窓部材を再び溶液中に沈める際、窓部材の表面には気泡が生じない。

８．　第６項において、防水用窓部材が液面に対して傾斜して配置されていることを特徴とする液浸走査型プローブ顕微鏡装置。

［効果］　窓部材が液面に対して傾斜しているので、窓部材に界面活性剤の被膜が良好に形成される。

【００３８】

【発明の効果】本発明によれば、探針とカンチレバーとカンチレバー保持部材に対する気泡の付着が少なくなるので、良好な走査が行なえるとともに、光学顕微鏡による観察も良好に行なえる。

【００３９】また、窓部材に対する気泡の付着が少なくなるので、正確な変位検出を行なえるようになる。さらに、界面活性剤は溶液中に混入しないので、生体試料に悪影響を与えることもない。

【図面の簡単な説明】

【図１】（Ａ）は本発明の第一の実施形態による液浸走査型プローブ顕微鏡の構成を示す図、（Ｂ）は（Ａ）のカンチレバー保持部材を拡大して示す図、（Ｃ）は（Ｂ）の１Ｃ－１Ｃ線によるカンチレバー保持部材の断面図である。

【図２】図１の液浸走査型プローブ顕微鏡における測定準備の手順を説明するための図である。

【図３】図２の手順に関連して、気泡が生じること無くカンチレバー保持部材が溶液中に沈められる様子を説明するための図である。

【図４】本発明の第二の実施形態による液浸走査型プローブ顕微鏡の構成を示す図である。

【図５】溶液中の試料を観察する走査型プローブ顕微鏡の全体の構成を示す断面図である。

【図６】（Ａ）は図５の探針保持部を下から見た図、（Ｂ）は探針保持部の断面図である。

【符号の説明】

１２…試料、１４…容器、１６…溶液、２０…探針、２

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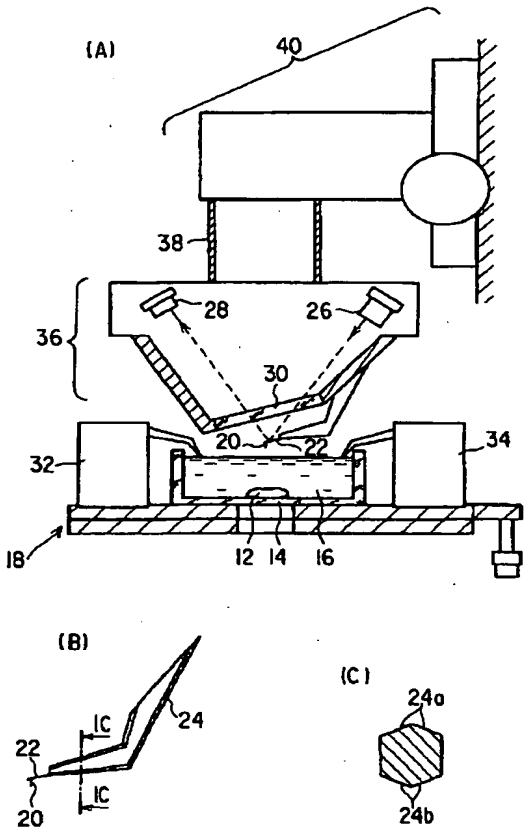
2…カンチレバー、２４…カンチレバー保持部材、２６…レーザー光源、２８…受光素子、３０…窓部材、３２

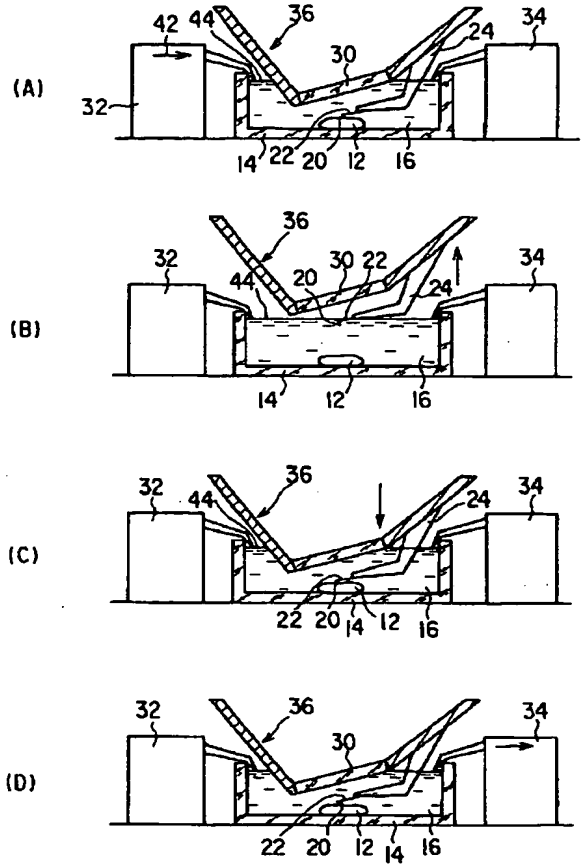
10

…マイクロディスペンサー、３６…ヘッド、４０…昇降手段。

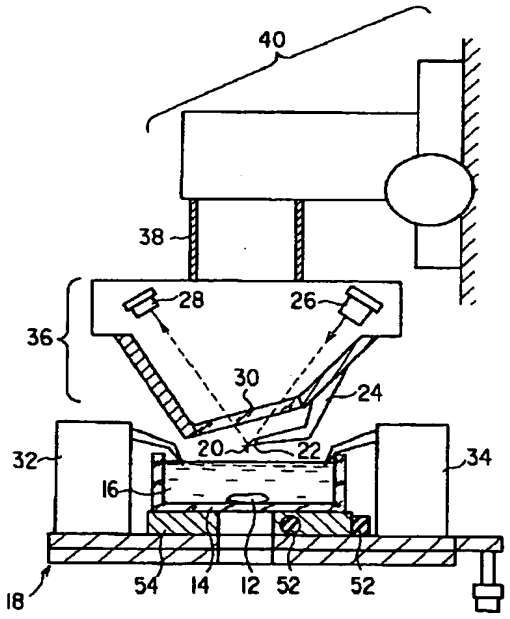
【図１】



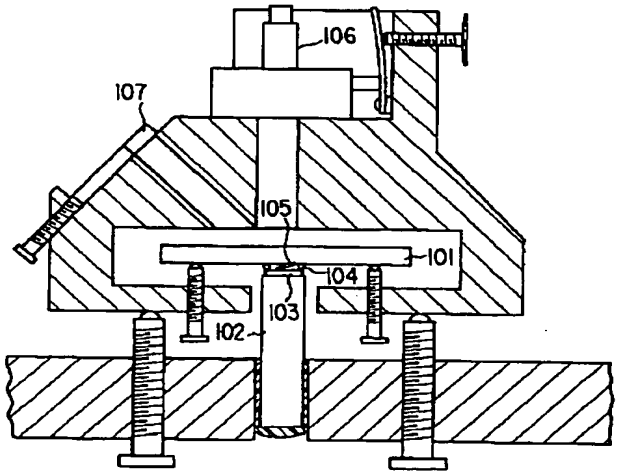
【図２】



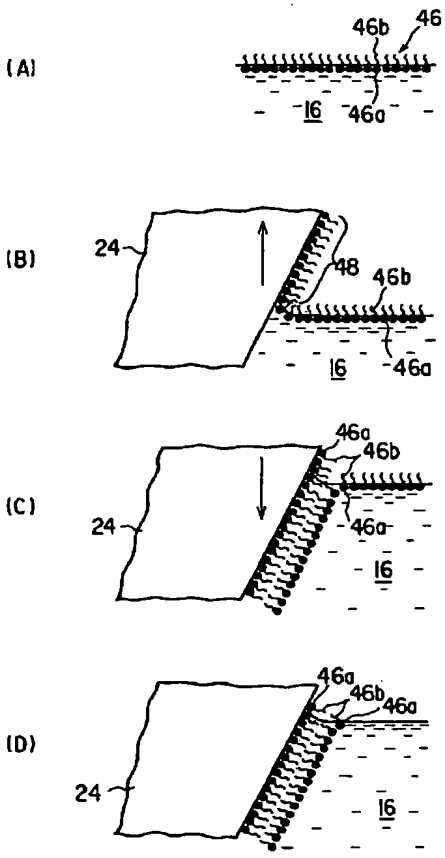
【図４】



【図５】



【図３】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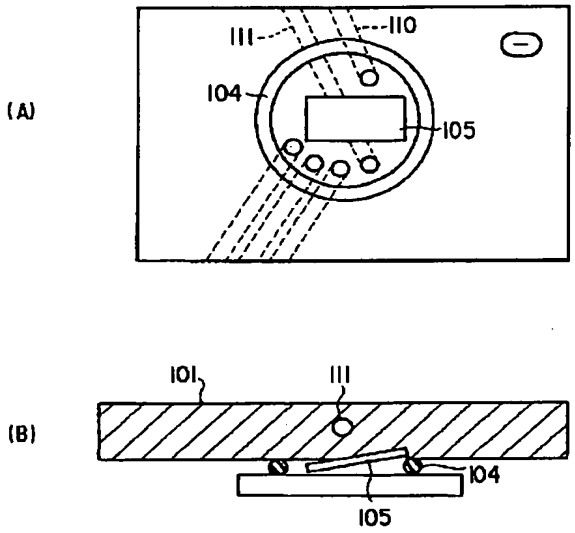
46
16
46a
(B)
24
48
46b
16
46a
(C)
46a
46b
24
46a
16
(D)
46a
46b
46a
24
16

【図６】


(A)
111
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(B)
101
111
104
105

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 09-119939

(43)Date of publication of application : 06.05.1997

(51)Int.Cl. G01N 37/00
G01B 21/30
H01J 37/28

(21)Application number : 07-277924

(22)Date of filing : 25.10.1995

(71)Applicant : OLYMPUS OPTICAL CO LTD

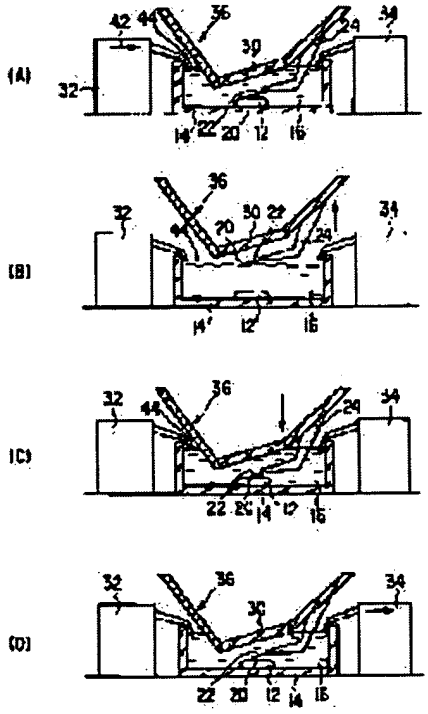
(72)Inventor : FUKUSHIMA NORICHIKA

(54) LIQUID-DIPPED SCANNING PROBE MICROSCOPE DEVICE

(57)Abstract:

PROBLEM TO BE SOLVED: To provide a liquid dipped scanning type probe microscope with less adhesion of air bubble to the surface of a member which is dipped in a solution.

SOLUTION: A probe 20, a cantilever 22, the entire portion of a window member 30, and one portion of a cantilever retention member 24 are sunk in a solution 16. After that, an interface activator is supplied onto the liquid surface of the solution 16 by a micro dispenser 32. Then, a head 36 is raised to such a height that the cantilever 22 and the probe 20 do not touch the activator on the liquid surface, thus adhering the interface activator to the cantilever retention member 24 and the window member 30. After that, the cantilever holding member 24 is sunk in the solution again. At this time, since the interface activator adhered to the cantilever holding member 24 and the window member 30 properly adapts itself to the interface activator on the liquid surface, no air bubble is generated on the surface. Finally, the interface activator remaining on the liquid surface is sucked by a collecting means 34.



LEGAL STATUS

[Date of request for examination]

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or application converted registration]

[Date of final disposal for application]

[Patent number]

[Date of registration]

[Number of appeal against examiner's decision of rejection]

[Date of requesting appeal against examiner's decision of rejection]

[Date of extinction of right]

* NOTICES *

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CLAIMS

[Claim(s)]
[Claim 1] In the immersion scan type probe microscope which observes the sample arranged in the solution put into the container using a probe The hydrophilicity--ization-processed cantilever which was equipped with the probe at the nose of cam, and the cantilever attachment component holding a cantilever, A means to carry out permeation shunting of a cantilever and the cantilever attachment component into a solution, Have a means to supply a surfactant, on the oil level of a solution, and observation of a sample is preceded. Sink the whole cantilever and a cantilever attachment component into a solution, supply a surfactant on the oil level of a solution, and the thin film of a surfactant is formed on an oil level. The immersion scan type probe microscope characterized by for a cantilever pulling up a cantilever attachment component if possible in the range which cannot touch a surfactant, and lowering a cantilever attachment component again.
[Claim 2] Have the following and a case has transparent window part material on the optical target which allows the traffic of the light between a displacement detection system and a cantilever. Window part material is the claim 1 which is aslant arranged to the oil level, sinks window part material into a solution in advance of observation of a sample, supplies a surfactant on the oil level of a solution, forms the thin film of a surfactant on an oil level, pulls up window part material, and is characterized by sinking window part material into a solution again. The displacement detection system which detects the variation rate of a cantilever optically. The case which holds a displacement detection system in order to protect a displacement detection system from a solution.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[The technical field to which invention belongs] this invention relates to scanned type probe microscope equipment.
[0002]
[Description of the Prior Art] A scanned type probe microscope is equipment which observes the detailed configuration of a sample by detecting the behavior of the probe which follows in accordance with a surface configuration during the scan of a sample, and the scanning tunneling microscope (STM), the atomic force microscope (AFM), the magnetic-force microscope (MFM), etc. are known by the classification of a means which a probe is made to follow.
[0003] for example, jogging whose cantilever or sample the probe which scans a sample front face is supported by the flexible cantilever in an atomic force microscope (AFM), and has jogging elements, such as a piezo electric crystal, -- a variation rate -- it is supported by the adjusting device and the interval of a probe and a sample can be adjusted If the attraction according to a van der Waals interaction to between the atom at the nose of cam of a probe and the atoms on the front face of a sample if a probe is brought close to a sample front face works and it brings close even to an atomic mutual distance grade further, the repulsive force by Pauli's exclusion principle will work shortly. Such attraction and repulsive force are named generically and called force between atoms. Although the force between atoms is very feeble, since the cantilever which is supporting the probe is very flexible, if the atom at the nose of cam of a probe receives the force between atoms, it will displace according to the size. A jogging displacement adjusting device carries out feedback control of the interval of a sample to a probe so that the amount of displacement of a cantilever may be kept constant. Since the voltage impressed to jogging elements, such as a piezo electric crystal, at the time of this feedback control corresponds to the configuration on the front face of a sample which the probe is scanning, the height information on the front face of a sample is acquired from this applied-voltage information, and the concavo-convex image on the front face of a sample is obtained by combining this with the positional information on a sample front face, and processing it.
[0004] If biological materials, such as a cell saved in solution using this scanned type probe microscope equipment, are observable, it is in a state [having lived unlike fixation of a sample like an electron microscope, or the observation under a vacuum], and since it becomes possible to perform the configuration of a sample, measurement of the physical properties of the front face in the analysis of the force which joins a probe, etc. with the resolution of several nm, development of usable scanned type probe microscope equipment is tried [that it is various and
[0005] The scanned type probe microscope currently indicated by U.S. JP,4935634,B as the typical example is shown in drawing 5 and drawing 6 . Drawing 5 is the cross section showing the composition of the whole scanned type probe microscope, and drawing and drawing 6 (B) as which drawing 6 (A) regarded the probe attaching part of drawing 5 from the bottom are the cross section of a probe attaching part.

[0006] As shown in drawing 5 , the probe attachment component 101 which supports a cantilever 105, and the sample base 103 prepared on the piezo electric crystal 102 touch the member 104 of the shape of a ring arranged among both, and the space surrounded by these members is filled with solution. As shown in drawing 6 , the exhaust pipe 111 for discharging with the supply pipe 110 for sending in a solution is formed in the ceiling portion of the probe attachment component 101 located inside the ring-like member 104. Supply of the oxygen for being immersed, and saving biological materials, such as a cell, by this, living and the temperature control of a solution are made possible. It returns to drawing 5 , and the sample placed on the sample base 103 is moved by the piezo electric crystal 102, and the probe prepared at the nose of cam of a cantilever 105 by this is scanned. The variation rate of a probe is detected during a scan by the displacement detection system constituted from a laser light source 106 which irradiates light, and a photo detector 107 which receives the reflected light by the upper surface of a cantilever 105.

[0007]

[Problem(s) to be Solved by the Invention] With the scanned type probe microscope equipment for immersion, a solution is poured into predetermined space like the upper example, or the cantilever equipped with a probe in the case of measurement is sunk into the solution accumulated in the specimen container. At this time, air bubbles may adhere to a cantilever or the front face of a cantilever attachment component. Removing is difficult for the once generated air bubbles. Such air bubbles become the obstacle of the microscope observation which serves as an obstacle of a scan and is used simultaneously and are not desirable.

[0008] About a probe and a cantilever, generating of air bubbles can be prevented by performing hydrophilicity-ized processing. Hydrophilicity-ized processing can be performed by performing oxidization plasma treatment or irradiating ultraviolet rays to the cantilever made from a silicon nitride. However, since a cantilever attachment component is generally made from a metal, it cannot perform hydrophilicity-ized processing.

[0009] Although mixing a surfactant into solution is generally known as a method of preventing adhesion of the air bubbles to the front face of the member in solution, when the sample which is a candidate for observation is a biological material, mixing of the surfactant to the inside of solution is not desirable on account of preservation of a sample.

[0010] Moreover, it is common that there are some which used the displacement detection system of an optical-lever method in the scanned type probe microscope equipment for immersion, and are the waterproof purpose and the transparent window part material for waterproofing is optically arranged between a cantilever and a displacement detection system in this case. In case the time of pouring in a solution and a probe are sunk also to this window part material for waterproofing, it is difficult for air bubbles to adhere and to also remove these air bubbles too. The air bubbles adhering to the aperture for waterproofing scatter the laser beam and the reflected light from a cantilever which go to the cantilever in the displacement detection system of an optical-lever method. This becomes the cause of a fall of displacement detection precision and is not desirable. Moreover, it becomes an obstacle for observation by the optical microscope used simultaneously and is not desirable. The purpose of this invention is that the adhesion of air bubbles to the front face of the member immersed into a solution offers a few immersion scan type probe microscope.

[0011]

[Means for Solving the Problem] In the immersion scan type probe microscope which observes the sample arranged in the solution which put this invention into the container using a probe The hydrophilicity--ization-processed cantilever which was equipped with the probe at the nose of cam, and the cantilever attachment component holding a cantilever, A means to carry out permeation shunting of a cantilever and the cantilever attachment component into a solution, Have a means to supply a surfactant, on the oil level of a solution, and observation of a sample is preceded. Sink the whole cantilever and a cantilever attachment component into a solution, supply a surfactant on the oil level of a solution, and the thin film of a surfactant is formed on an oil level. If possible, a cantilever pulls up a cantilever attachment component in the range which cannot touch a surfactant, and is characterized by

lowering a cantilever attachment component again.
[0012] In the above-mentioned composition, it has the displacement detection system which detects the variation rate of a cantilever optically, and the case which holds a displacement detection system in order to protect a displacement detection system from a solution. furthermore, a case It has transparent window part material on the optical target which allows the traffic of the light between a displacement detection system and a cantilever. It is characterized by window part material being aslant arranged to the oil level, sinking window part material into a solution in advance of observation of a sample, supplying a surfactant on the oil level of a solution, forming the thin film of a surfactant on an oil level, pulling up window part material, and sinking window part material into a solution again.
[0013]
[Embodiments of the Invention] Hereafter, the gestalt of operation of this invention is explained, referring to a drawing.
<the gestalt of the first operation> -- the gestalt of the first operation is first explained using drawing 1 - drawing 3
[0014] As shown in drawing 1 (A), a sample 12 is put on the pars basilaris ossis occipitalis of a container 14, and the solution 16 for a sample 12 being immersed is filled by the container 14. A container 14 is placed on mechanical X-Y stage 18, and the sample 12 to a probe 20 can be positioned. On X-Y stage 18, the micro dispenser 32 which is a means to supply a surfactant to the oil level of a solution 16 at the both sides of a container 14, and a recovery means 34 to collect the surfactants supplied to the oil level are established.
[0015] As shown in drawing 1 (B), the probe 20 is formed at the nose of cam of a cantilever 22, and a cantilever 22 is attached in the cantilever attachment component 24, and is supported. The hydrophilic property of ten or less contact angles is attained by a probe 20 and a cantilever 22 using a silicon nitride for the quality of the material, and performing 10-second room [about] oxidization plasma treatment. Hydrophilic processing of the front face of a silicon nitride is obtained also by UV irradiation. As for the cantilever attachment component 24, in the cross-section configuration, as shown in drawing 1 (C), upper surface 24a and inferior-surface-of-tongue 24b serve as a V character configuration. As the cantilever attachment component 24 is shown in drawing 1 (A), it is attached in the head 36 removable, and improvement in workability at the time of exchanging a cantilever 22 is achieved. Moreover, the excitation means which used the piezoelectric device near the cantilever 22 of the point of the cantilever attachment component 24 is attached, and you may perform the so-called AFM measurement in AC mode measured not applying external force to a sample, vibrating a cantilever 22 with a fixed amplitude.

[0016] The transparent window part material 30 is optically attached in the portion to which a head 36 is located above a cantilever 22 aslant, the whole is sealed and a solution permeates. The laser light source 26 which irradiates a laser beam on the upper surface of a cantilever 22, and the photo detector 28 which receives the reflected light from the upper surface of a cantilever 22 are held in the interior of a head 36. The photo detector 28 has two or more light-receiving fields which show the output according to the amount of incident lights, and the variation rate of a cantilever 22 is called for from the position of the spot of the reflected light formed on the light-receiving field of a photo detector 28.
[0017] The head 36 is supported by the cylindrical piezo electric crystal 38, and the cylindrical piezo electric crystal 38 is attached in the rise-and-fall means 40. Movement of the horizontal direction for XY scan and Z position control and a perpendicular direction is possible for a head 36 by the cylindrical piezo electric crystal 38. Moreover, in case rough ** of a head 36 has become possible up and down by the rise-and-fall means 40, for example, a container 14 is exchanged, in order to secure the space for taking a container 14 in and out, it shunts up.
[0018] Hereafter, the procedure of sinking a cantilever 22 and the cantilever attachment component 24 into a solution 16 is explained using drawing 2 and drawing 3 . First, a head 36 is dropped using the rise-and-fall means 40, and as shown in drawing 2 (A), a part of a probe 20, cantilever 22, whole window part material 30, and cantilever attachment component 24 are sunk into a solution 16. Since the probe 20 and the cantilever 22 are hydrophilicity--ization-processed, air bubbles do not adhere to these. Then, the

surfactant of the specified quantity is supplied on the oil level of a solution 16 using the micro dispenser 32, and the monomolecular film 44 of a surfactant 42 is formed in an oil level. The amount of the surfactant 42 to supply is a complement and it is decided in the area of opening of a container 14 that it forms a monomolecular film in an oil level. As shown in drawing 3 (A) at this time, hydrophilic-group 46a is turned to a solution 16 side, and each molecule 46 of the surfactant of an oil level turns hydrophobic-group 46b to an atmosphere side, and is located in a line with an oil level. A barium stearate is raised as a surfactant to be used.

[0019] Next, using the rise-and-fall means 40, as shown in drawing 2 (B), a cantilever 22 and a probe 20 raise a head 36 to the height which cannot touch the monomolecular film 44 of a surfactant. At this time, almost all the portions of the whole window part material 30 and the cantilever attachment component 24 can pull up in the atmosphere. When the cantilever attachment component 24 can pull up from a solution 16, hydrophilic-group 46a is turned to the front face of the cantilever attachment component 24, and the molecule 46 of the surfactant on an oil level adheres to it, as shown in drawing 3 (B). Although only the cantilever attachment component 24 is shown in drawing, similarly, hydrophilic-group 46a is turned also to the front face of the window part material 30, and it adheres to it. Consequently, the coat 48 of the surfactant with which hydrophobic-group 46b was suitable into the atmosphere is formed in the front face of the cantilever attachment component 24 and the window part material 30. Since the upper surface and the inferior surface of tongue of the cantilever attachment component 24 serve as a V character configuration as shown in drawing 1 (C), the coat 48 of a surfactant is formed also in the upper surface and an inferior surface of tongue good. Since hydrophilicity-ized processing is performed at this time, as for a probe 20 and a cantilever 22, it is important that it is careful so that the coat of a surfactant may not be formed in these front faces.

[0020] In addition, as shown in drawing 2 , as for the cantilever attachment component 24, it is desirable to incline and constitute to the oil level of a solution 16 so that it may infiltrate into a solution 16 gradually in the direction of the end face section from the point of a cantilever 22. Thus, by constituting, the cantilever attachment component 24 can be hydrophilicity--ization-processed effectively, without making a surfactant touched with a cantilever 22.

[0021] Then, a head 36 is dropped using the rise-and-fall means 40, and as shown in drawing 2 (C), a probe 20 is close brought to near the front face of a sample 12. Since the coat of the surfactant with which hydrophobic-group 46b was outside suitable is formed in the front face of the cantilever attachment component 24 and the window part material 30 at this time and there is a monomolecular film of the surfactant which turned hydrophobic-group 46b to the atmosphere side in an oil level In case the cantilever attachment component 24 and the window part material 30 sink into a solution again, as shown in drawing 3 (C) The surfactant on an oil level adheres to the coat of the surfactant of the front face of the cantilever attachment component 24 and the window part material 30 good, and the coat of the surfactant of a bilayer eye with which hydrophilic-group 46a was outside suitable is formed. Consequently, the cantilever attachment component 24 and the window part material 30 are sunk into a solution, without a foam adhering to a front face.

[0022] Finally, as shown in drawing 2 (D), the surfactant which remains on an oil level from the nozzle which touched the oil level is attracted using the recovery means 34. Consequently, as shown in drawing 3 (D), surfactants are collected except for the circumference of the member immersed into the cantilever attachment component 24, the window part material 30, and the solution. Now, preparation of AFM measurement is completed.

[0023] <The form of the second operation>, next the form of the second operation are explained using drawing 4 . On X-Y stage 18, the jogging stage 54 moved in the XY direction is formed by two piezo electric crystals 52, and the container 14 into which the sample 12 and the solution 16 were put is arranged on the jogging stage 54.

[0024] A sample 12 is moved in the XY direction by the jogging stage 54 in the case of a scan. Therefore, the cylindrical piezo electric crystal 38 only performs flexible operation for moving a head 36 to a Z direction, and does not perform curve operation for moving this in the XY direction.

[0025] Other composition and operations are the same as the form of the first operation of the above-

mentioned. With the form of this operation, the cylindrical piezo electric crystal 38 is allowed to enlarge the outer diameter, since curve operation for movement in the XY direction is unnecessary. By enlarging the outer diameter of a cylindrical piezo electric crystal, it is possible to arrange a large-sized condensing lens with big numerical aperture advantageous to the observation for optical microscopes etc. inside.

[0026] this invention is not limited to the form of above-mentioned operation at all. All operations performed in the range which does not deviate from the summary of invention are included in this invention. Invention described in each following term is included in this specification.

[0027] 1. For Example, Sample Front Face Fixed in Containers, Such as Petri Dish with which Solutions, Such as Physiological Saline, were Filled In the immersion scan type probe microscope equipment which acquires information, such as a configuration of a sample, by scanning by the probe concluded by the probe attachment component inserted into the solution The monomolecular film of the matter (surfactant) which has the insoluble surface activity effect in water Immersion scan type probe microscope equipment characterized by forming on the oil level of the solution filled in the container, and forming a coat in the front face of a cantilever attachment component using own adsorptivity of a surfactant in case a probe is introduced into a solution.

[0028] [Effect] Since generating of the air bubbles of the front face of the probe attachment component introduced into liquid when the molecule of a surfactant turns a hydrophilic group outside and forms a coat in the front face of the probe attachment component introduced into the solution can be reduced, the scanned type probe microscope equipment in which the stable observation operation is possible is offered.

[0029] 2. Immersion scan type probe microscope equipment characterized by having process of operation which forms hydrophilic coat in cantilever attachment component front face by pulling up only probe attachment component introduced into solution out of the atmosphere from oil level, and introducing it into solution again this back at once in the 1st term in process in which coat of surfactant is made to form in front face of cantilever attachment component.

[0030] [Effect] A surfactant molecule does not adhere at the nose of cam of a probe, but degradation of the quality of image of the scanning picture which considers adhesion of the foreign matter to the nose of cam of a probe as a cause is prevented.

3. Immersion scan type probe microscope equipment characterized by the upper surface and inferior surface of tongue of cross-section configuration of cantilever attachment component having V character configuration in the 1st term.

[0031] [Effect] Since the cross-section configuration of the upper surface and the inferior surface of tongue of a cantilever attachment component is a V character type, coat formation of a surfactant on the attachment component front face at the time of passing through the thin film side of the surfactant formed on the oil level of a solution becomes easy, and the scanned type probe microscope equipment which was excellent in the reduction effect of gassing by the surfactant is obtained.

[0032] 4. Immersion scan type probe microscope equipment characterized by having means to supply surfactant on oil level of solution, in the 1st term or the 3rd term.

[Effect] Since it has the supply means of a surfactant, the scanned type probe microscope equipment excellent in operability which does not need to prepare supply meanses, such as a dispenser, separately and can form them easily on the oil level of a solution at every observation operation of the thin film of a surfactant is obtained.

[0033] 5. Immersion scan type probe microscope equipment characterized by having means to collect surfactants on oil level of solution, in the 1st term or the 4th term.

[Effect] Since it has the recovery means of a surfactant, when it can collect the thin films of the surfactant formed on the oil level of a solution and makes a solution flow back for preservation of the biological material under observation, possibility that the surfactant on an oil level will reach a sample is reduced. Moreover, since mixing into a solution is prevented to the surfactant at water, such as a barium stearate, using the insoluble matter, scanned type probe microscope equipment excellent in sample protection nature is obtained.

[0034] 6. Monomolecular Film of Matter (Surfactant) Which Has the Insoluble Surface Activity Effect in Water In case it forms on the oil level of the solution filled in the container and a probe is introduced into a solution For the purpose of protection of displacement detection meanses, such as for example, an optical-lever method allotted to the cantilever upper part in order to detect the variation rate of a cantilever, between a cantilever and a displacement detection means Immersion scan type probe microscope equipment characterized by forming a coat in the front face of the window part material for waterproofing allotted using own adsorptivity of a surfactant.
[0035] [Effect] Since generating of the foam of the front face of the window part material introduced into liquid when the molecule of a surfactant turns a hydrophilic group outside and forms a coat in the front face of the window part material introduced into the solution can be reduced, the scanned type probe microscope equipment in which exact displacement detection is possible is offered.
[0036] 7. Immersion scan type probe microscope equipment characterized by having process of operation which forms coat of hydrophilic property in front face of window part material for waterproofing by pulling up only probe attachment component introduced into solution out of the atmosphere from oil level, and introducing it into solution again this back at once in the 6th term in process in which coat of surfactant is made to form in front face of window part material for waterproofing.
[0037] [Effect] Since the surfactant adhering to window part material and the surfactant on an oil level get used well, in case they sink window part material into a solution again, in the front face of window part material, a foam does not produce them.
8. Immersion scan type probe microscope equipment characterized by for window part material for waterproofing inclining and arranging it to oil level in the 6th term.
[Effect] Since window part material inclines to an oil level, the coat of a surfactant is formed in window part material good.
[0038]
[Effect of the Invention] Since the adhesion of a foam to a probe, a cantilever, and a cantilever attachment component decreases, while being able to perform a good scan according to this invention, observation by the optical microscope can also be performed good.
[0039] Moreover, since the adhesion of a foam to window part material decreases, exact displacement detection can be performed. Furthermore, since a surfactant is not mixed into a solution, it does not have a bad influence on a biological material.

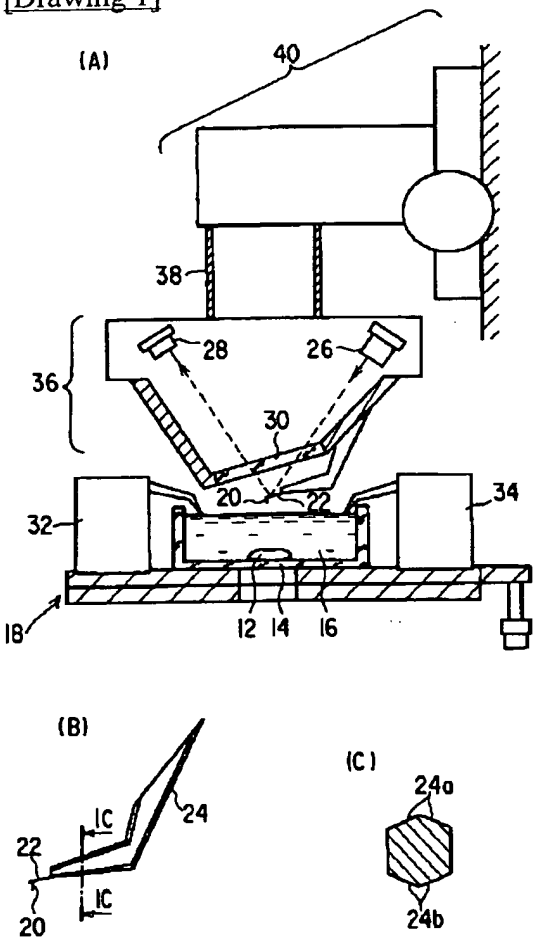
[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

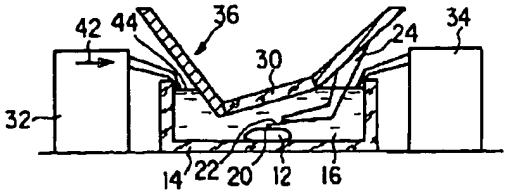
DRAWINGS

[Drawing 1]



[Drawing 2]

(A)



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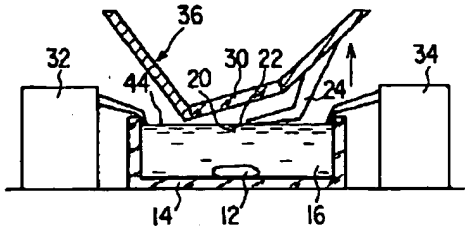

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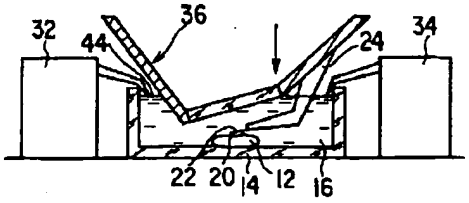

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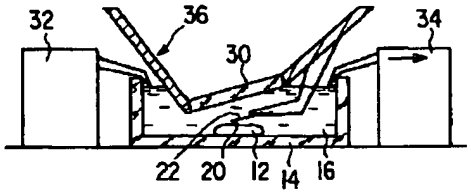
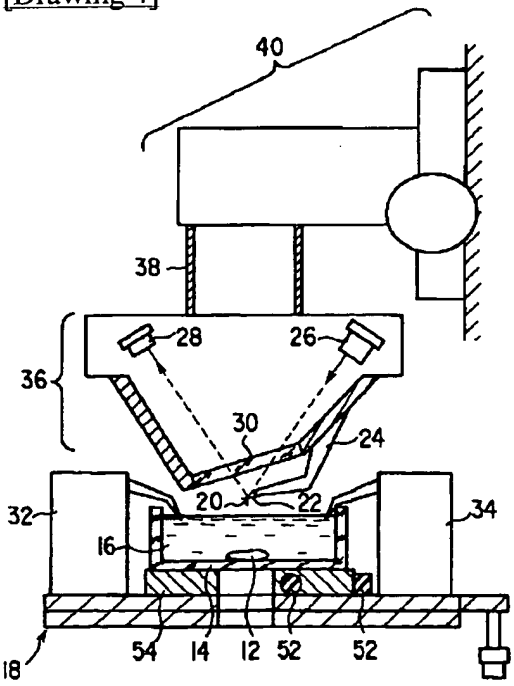
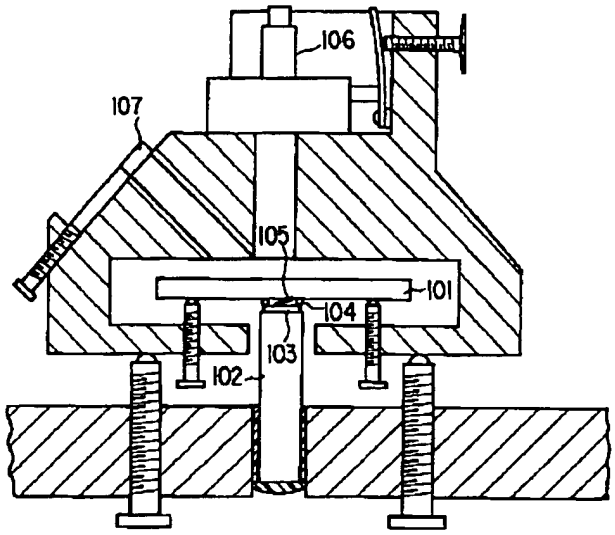


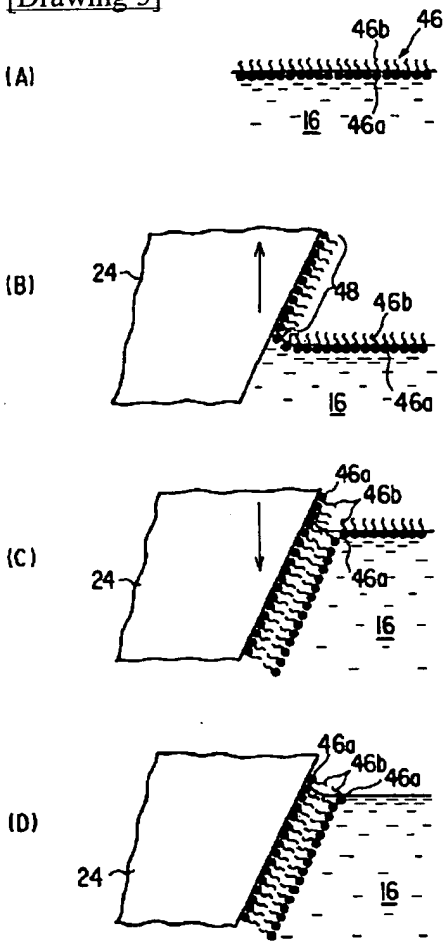
[Drawing 4]



[Drawing 5]

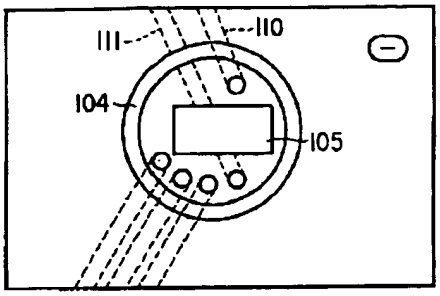


[Drawing 3]



[Drawing 6]

(A)



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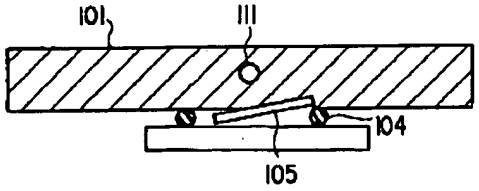

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